

Anti-V5 and Anti-V5-HRP Antibody

Cat. Nos. R960-25, R961-25
Conc. Refer to the label on the tube

Size: 50 µl
Store at 4°C

Introduction

The Anti-V5 Antibody allows detection of recombinant proteins containing the V5 epitope. This epitope is found in the P and V proteins of the paramyxovirus, SV5 (Southern *et al.*, 1991). The Anti-V5 Antibody recognizes the 14 amino acid sequence:

Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr

Detailed protocols for using the antibody are in the technical manual available from our website at www.invitrogen.com. or in published references (Ausubel *et al.*, 1994; Harlow & Lane, 1988).

Contents

The Anti-V5 Antibody (R960-25) is a mouse monoclonal IgG_{2a} antibody. Anti-V5-HRP Antibody (R961-25) is prepared by crosslinking the Anti-V5 Antibody with horseradish peroxidase (HRP) using glutaraldehyde. Sufficient antibody (50 µl) is supplied to perform 25 western immunodetections (assuming 10 ml buffer per blot).

Specifications

Storage Buffer: 1X phosphate buffered saline (PBS) with 0.01% sodium azide

Storage: Store at 4°C. For long-term storage, aliquot the antibody and store at -20°C or -80°C. Avoid repeated freezing and thawing.

Stability: 6 months at 4°C

Part No. R960.pps

Rev. Date: 23 Jan 2007

Antibody Specificity

Both antibodies have been tested in immunoblotting and ELISA procedures. Low background was observed using chemiluminescent or alkaline phosphatase reagents for detection.

In western blot experiments with purified protein, 25 ng (for Anti-V5 Antibody) or 50 ng (for Anti-V5-HRP Antibody) of recombinant Positope™ protein (Catalog no. R900-50) gave a detectable signal using a standard immunoblotting protocol (refer to the technical manual available from www.invitrogen.com).

The Anti-V5 Antibody has also been used successfully to immunoprecipitate fusion proteins that contain the V5 epitope.

Crossreactivity

Using chemiluminescence as the detection method, no crossreactivity is observed in bacterial lysates. In mammalian lysates, a few crossreactive proteins are observed upon overexposure of blots.

Recommended Dilutions

We recommend the following dilutions of the supplied antibody for these applications:

- For western blots, dilute 1:5000 into PBS or Tris-Buffered Saline (TBS) containing 0.05% Tween-20 and 5% nonfat, dry milk (PBSTM or TBSTM).
- For ELISA assays, serially dilute into PBSTM or TBSTM and test various dilutions to determine the best antibody dilution for your application.

If you use a different buffer for washing and blocking your blots, dilute as described above with that buffer. You may use other blocking agents such as bovine serum albumin (BSA) or gelatin.

Important

If you use azide in your HRP-conjugated antibody dilution buffers, wash the western blot or microtiter wells thoroughly before adding the color development solution. Azide inhibits HRP activity.

If you use alkaline phosphatase-conjugated secondary antibody, do not use PBS. Phosphate inhibits alkaline phosphatase. Use TBS instead.

Protocols

Detailed protocols for western and dot blotting, ELISA, immunoprecipitation, and immunostaining are described in the technical manual available from our website at www.invitrogen.com or contact Technical Support.

Product Qualification

The Anti-V5 Antibody and the Anti-V5-HRP Antibody are functionally tested by western blot as follows:

Anti-V5 Antibody

The antibody must react specifically with 50 ng of an *E. coli* expressed fusion protein containing a V5 epitope. Western blots must reveal a strong signal, with no nonspecific background, after a 5-minute development with a chromogenic substrate.

Anti-V5-HRP Antibody

The antibody must react specifically with 20 ng of an *E. coli* expressed fusion protein containing a V5 epitope. Western blots must reveal a strong signal, with no nonspecific background, after development with a chemiluminescent substrate followed by a 1 minute exposure to x-ray film.

References

- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994) *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley-Interscience, New York
- Harlow, E., and Lane, D. (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Southern, J. A., Young, D. F., Heaney, F., Baumgartner, W., and Randall, R. E. (1991) Identification of an Epitope on the P and V Proteins of Simian Virus 5 That Distinguishes Between Two Isolates with Different Biological Characteristics. *J. Gen. Virol.* 72, 1551-1557

Accessory Products

A large variety of pre-cast gels, immunodetection kits, and ELISA reagents is available from Invitrogen for use with the Anti-V5 Antibody and Anti-V5-HRP Antibody. For details on these products, visit

www.invitrogen.com/antibody.

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